VACCINES AGAINST CHLAMYDIA SP.

FIELD OF INVENTION

[0001] The present invention relates to polypeptides of repetitive units of immunogenic fragments of surface exposed regions of outer membrane proteins of *Chlamydia* sp. and pharmaceutical compositions and vaccines comprising these fusion proteins.

BACKGROUND OF THE INVENTION

[0002] Chlamydiae are intracellular bacterial pathogens responsible for a variety of infections. *Chlamydia pneumoniae* is responsible for human acute respiratory infection and believed to play a role in coronary heart disease. *Chlamydia trachomatis* is the causative agent of human sexually transmitted disease and eye infections (Trachoma). Also in animals, several infections with *Chlamydia* sp. are known, e.g. *Chlamydia Suis* infecting pigs, and *Chlamydiaphila abortus* which causes abortion in small ruminants (sheep and goats).

[0003] Worldwide, it is estimated that 92 million individuals become sexually infected with *Chlamydia trachomatis* (Ct)[1]. Urogenital infections with Ct are of public health concern because of its high prevalence and the fact that it's a risk factor for ectopic pregnancy and infertility[2]. In addition to this Ct infections have been shown to facilitate the transmission of HIV[3] and act as a co-factor in HPV-induced cervical carcinoma[4]. The duration of untreated genital Ct infection can be prolonged, and complete clearance is often not reached within the first 12 months[5]. From human studies it is known that some degree of protective immunity against genital re-infection develops, although it appears at best to be partial[6]. The infection is effectively controlled by antibiotic therapy; however the high prevalence of asymptomatic cases suggests that sustainable disease control can only be envisaged if an effective *Chlamydia* vaccine is developed.

[0004] A vaccine against Ct needs to elicit protective T-cell and B-cell immunity in the genital tract mucosa[7]. Immune mechanisms of clearance of infection and resistance to re-infection have been described in numerous studies. A variety of animal models and chlamydial species have been used in attempts to identify protective and damaging immune responses. A general consensus has emerged that, in mice, $CD4^+$Th1 cell mediated immune responses plays a major role in the resolution of Ct infection[8, 9, 10], whereas the role of humoral immunity in protection has remained less well defined. In guinea pigs immunity to chlamydial infection is mediated at least partly by secretory IgA at the mucosal surface[11, 12] and also in the mouse model there is increasing evidence to support a role for antibodies in protective immunity[9]. Data from animal models that has emerged over the last years clearly demonstrate that if antibodies are formed after the infection is established they play a minimal role, whereas their presence at the time of infection (e.g. in a secondary response) promotes significant levels of protection, an effect that is however clearly amplified in the presence of *Chlamydia* specific $CD4^+$ cells[9, 13, 14]. A strong cell mediated immune (CMI) response without antibodies may on the other hand control bacterial replication but can in the worst case exacerbate the pathology associated with *Chlamydia* infection[15 16]. The importance of this interplay between cell mediated immunity and antibodies is also becoming increasingly clear to support a preferential role of neutralizing antibodies in the initial phase of infection, whereas $CD4^+$ cells are the main effectors throughout the rest of the infection[17 18 19]. In summary balancing the immune effector mechanisms between antibodies and T cells seems to be crucial for disease outcome.

[0005] We and others have identified a range of chlamydial antigens recognized during a natural infection in either humans or animal models[20, 21 22, 23 24 25, 26 27]. Especially the publishing of the genome sequence in 1998 and modern high throughput techniques have led to the testing of almost the entire genome of 875 open reading frames[28]. Importantly, identifying proteins as antigenic during an infection do not necessarily mean they are protective as vaccines[29] and despite the characterization of such a large number of antigens only very few of these have been demonstrated to mediate protection as vaccines in animal models[30 31, 32]. Furthermore for the majority of the vaccines recently reported the partial protection observed is mediated by T cells with no neutralizing antibodies. Therefore there is a lack of vaccine candidates that generate neutralizing antibodies that can cope with the infection in the initial phase and creating a balanced immune response.

[0006] Until now there has only been convincing data on neutralizing antibodies with three surface exposed antigens; PorB, which localized in the chlamydial outer membrane and functions as a porin[33]. Antibodies against this has been shown to neutralize chlamydial infectivity[34], patent ref: U.S. Pat. No. 7,105,171. Another more recent antigen is PmpD. This protein has been shown to generate neutralizing antibodies in vitro, however the in vivo relevance of these antibodies have not yet been demonstrated[35].

[0007] MOMP is the classical target antigen for neutralizing antibodies and one of the first antigenic molecules described. It is a surface-exposed trans membrane protein which has structural (porin) properties[36, 37, 38] MOMP is a 40 kDa protein making up roughly 60% of the protein in the Ct membrane and is a target for neutralizing antibodies with proven efficacy both in vitro and in vivo. MOMP consists of four variable surface exposed domains (VD-1 to VD-4) separated by five constant segments[36 39] and it is the molecular basis of the serovar (~15) grouping of *Chlamydia* (FIG. **1**). The in vitro and in vivo neutralizing antibody epitopes have been mapped to these VDs[40 41 42 43 44]. The distribution profile of Ct urogenital serovars has been described for regions worldwide, providing epidemiological data for the serovar coverage needed of a MOMP based vaccine. The most common serovar detected worldwide is E (22-49% of cases) followed by serovars F and D (17-22% and 9-19%, respectively)[45 46 47 48 49 50] meaning that a vaccine targeting serovars E, D and F would have a significant impact and cover more than 70% of the human population.

[0008] MOMP is highly immunogenic in humans and animals and has therefore been studied in great detail as a vaccine candidate, both as a natively purified protein, recombinantly and as DNA-vaccine. These vaccination attempts gave variable results[17, 51, 52, 53, 54, 55, 56, 57]. The reason for the relative inconsistency of MOMP as a vaccine is not fully understood, but the fact that the synthetic MOMP immunogens do not mimic the native structure of the protein has been the major concern[54]. In this regard, the structure of this membrane bound cysteine rich molecule and refolding various products to achieve native protein structure has been extremely challenging and is not suitable for large scale vaccine production[58]. Therefore, although clearly with vaccine potential, full size MOMP has so far not been a feasible